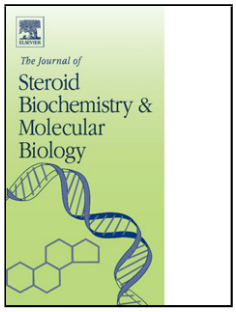

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Impact of vitamin D supplementation on endothelial and inflammatory markers in adults: A systematic review.

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Highlights

- Vitamin D deficiency has been identified as a potential risk factor for cardiovascular disease.
- Review of RCTs of effects of vitamin D supplementation on endothelial function/inflammation.
- 8/29 studies reported improvements in the endothelial/inflammatory parameters measured.
- This review does not support use of vitamin D as a preventative measure for CVD.

Abstract

This systematic review aims to evaluate randomised controlled trials (RCTs) investigating the effect of vitamin D supplementation on endothelial function and inflammation in adults. An electronic search of published randomised controlled trials, using Cochrane, Pubmed and Medline databases was conducted, with the search terms related to vitamin D and endothelial function. Inclusion criteria were RCTs in adult humans with a measure of vitamin D status using serum/plasma 25(OH)D and studies which administered the intervention through the oral route. Among the 1107 studies retrieved, 29 studies met the full inclusion criteria for this systematic review. Overall, 8 studies reported significant improvements in the endothelial/inflammatory biomarkers/parameters measured. However, in 2 out of the 8 studies, improvements were reported at interim time points, but improvements were absent post-intervention. The remaining 21 trial studies did not show significant improvements in the markers of interest measured. Evidence from the studies included in this systematic review did not demonstrate that vitamin D supplementation in adults, results in an improvement in circulating inflammatory and endothelial function biomarkers/parameters. This systematic review does not therefore support the use of vitamin D supplementation as a therapeutic or preventative measure for CVD in this respect.

Key Words: Vitamin D; 25(OH)D; endothelial function; inflammation; flow mediated dilation; Randomized controlled trials.

1. **Introduction**

Vitamin D is a fat soluble secosteroid hormone with both endocrine and autocrine functions [1]. The primary endocrine function of vitamin D is the maintenance of calcium homeostasis and bone metabolism which is achieved through the modulation of intestinal and kidney calcium absorption [2] and reabsorption from the bones [3]. The autocrine function of vitamin D depends on genetic transcription unique to the cell type expressing the vitamin D receptor (VDR). One such autocrine effect is the modulation of inflammatory pathways which play a role in cardiovascular diseases (CVD) amongst others [4, 5].

Vitamin D deficiency is defined by a shortage of the active vitamin D metabolite calcitriol in target cells [6]. Vitamin D deficiency/insufficiency may raise CVD risk by activating a pro-inflammatory cascade which may cause a rise in arterial stiffness and endothelial dysfunction, which are well known surrogates of CVD risk [7]. One study which compared vitamin D deficient subjects with those in the sufficient range, concluded that subjects in the deficient group showed double the risk of myocardial infarction [8]. Interventions involving vitamin D supplementation have shown improvement in biomarkers of endothelial dysfunction and inflammation, including one intervention which reported a significant decrease in the endothelial biomarker E- selectin after administering 300,000 IU of vitamin D_3 at baseline and eight weeks in 26 non-diabetic patients with chronic kidney disease (CKD) [9]. Another study which supplemented with 2000 IU of vitamin D_3 over 9 months in 123 patients with congestive heart failure, reported a significant increase in plasma concentrations of the anti-inflammatory cytokine, interleukin 10 (IL-10) [10].

The endothelium plays an important function in maintaining vascular health. It regulates vascular tone, and modulates haemostasis and inflammation by several mechanisms. These include; the production of nitric oxide (NO) and prostacyclin, which exert anti-aggregatory effects on platelets, and the secretion of heparin and protein C/S (vitamin K- dependent plasma proteins) which exert anti-coagulatory or fibrinolytic properties, and the inhibition of vascular growth [11,12].

To our knowledge, there are currently no systematic reviews investigating whether vitamin D supplementation provides an enhanced anti-inflammatory response and improves endothelial function. The aim of this review is to evaluate randomized placebo-controlled

trials investigating the effects of vitamin D on endothelial function and inflammatory markers in adults. It is hypothesized that vitamin D supplementation in adults will lead to a reduction in circulating inflammatory and endothelial function biomarkers, thereby proposing a potential role for vitamin D as an anti-inflammatory therapy for the prevention and treatment of CVD.

2. Methods

2.1 Study selection

A systematic literature search of the Cochrane, Pubmed and Medline electronic databases was conducted for articles published from 2008 to 2014. The following search terms were used to search for relevant publications: “VITAMIN D AND ENDOTHELIAL FUNCTION”, “VITAMIN D AND ENDOTHELIAL FUNCTION BIOMARKERS”, “VITAMIN D AND CARDIOVASCULAR DISEASE’’, “VITAMIN D AND INFLAMMATORY BIOMARKERS’’ and “VITAMIN D AND INFLAMMATORY CYTOKINES”. The search was repeated substituting “VITAMIN D” for “CHOLECALCIFEROL” for the above search terms. See Fig 1 & Table 1.

2.2 Inclusion criteria

Studies were included in the systematic review if they fulfilled the following criteria:

- Randomized, double-blind, placebo-controlled trials in adult humans.
- Measure of vitamin D status using serum or plasma 25(OH)D.
- Supplemented with vitamin D through the oral route of administration (excluding fortified drinks and food), as it has been reported that vitamin D administration through the oral route produces higher peak 25(OH)D concentrations, and has a longer duration of effect, than the intramuscular route [13].

2.3 Exclusion criteria

Studies were excluded if:

- Participants were younger than 18 years of age.

- Vitamin D was also combined with calcium (these were excluded to minimize confounding).
- Studies that used vitamin D analogues.

2.4 Screening of articles for eligibility

Included studies were selected from electronic databases according to the search criteria. Title and abstracts obtained were screened for relevant articles. Articles not relevant to the systematic review objectives, outcome of interest, used an alternative study design and were duplicate publication and/or were not published in English were discarded.

2.5 Data collection and extraction

The following data was extracted from the selected studies: first author, country and year of study, study participants, gender, sample size, baseline 25(OH)D and post intervention 25(OH)D concentration, duration and dose of the intervention and baseline and post intervention endothelial function outcome measures. The relevant statistically significant *P* values were recorded. The methodological quality of each RCT was assessed using Jadad scale for reporting RCTs [14] .

2.6 Synthesis of results

The results were synthesized by constructing a descriptive summary of the included studies in table form (Table 1). The risk of bias was narrated and is based on: randomization, blinding and use of a placebo group. Intervention outcome measures are presented as mean ± standard deviation (SD). Median values were used for assessment where mean values were not reported. For all values, $P \leq 0.05$ was considered statistically significant. The PRISMA (2009) checklist was followed to structure the systematic review.

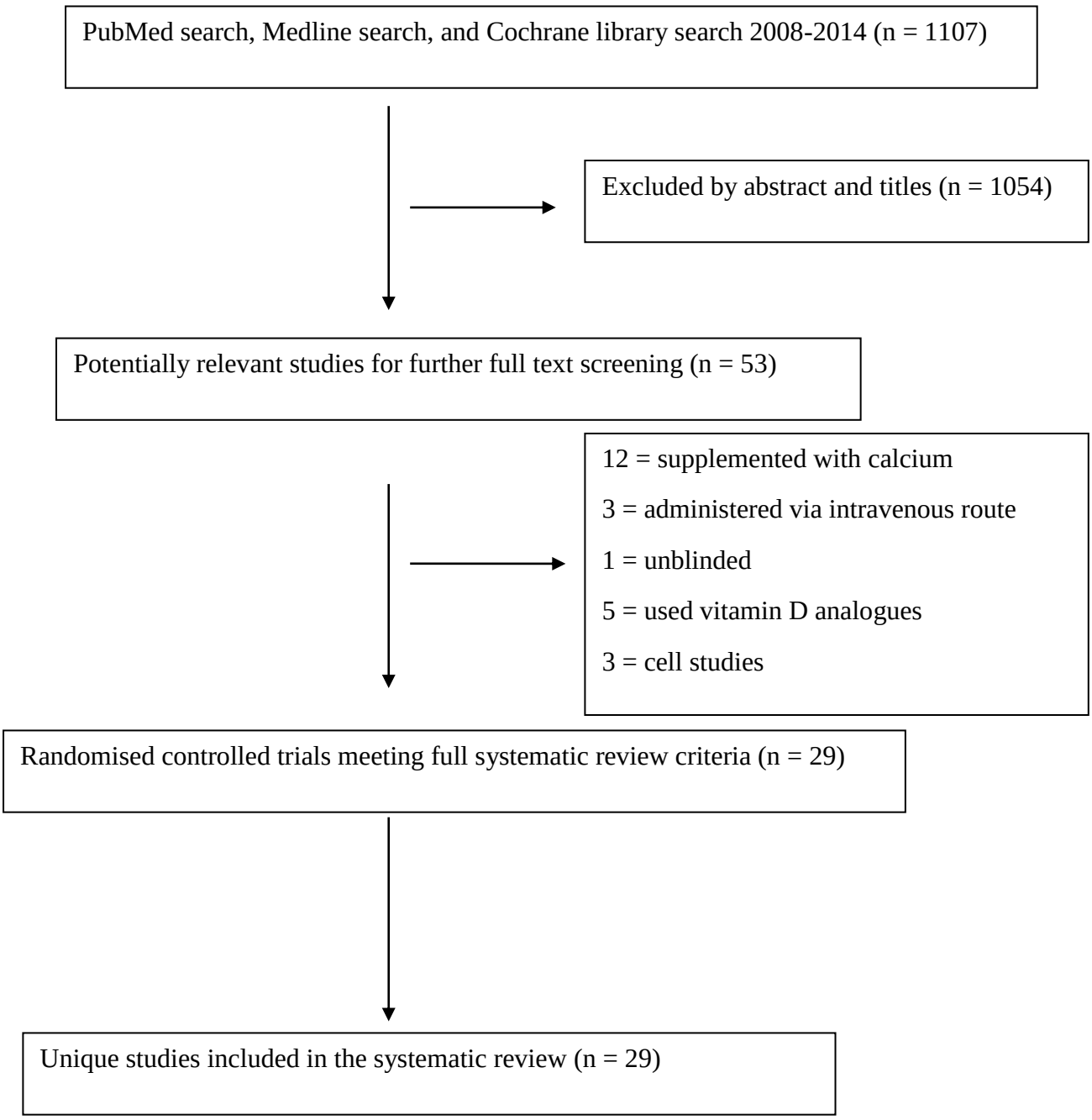

Figure 1. Flow chart of included studies

3. Results

On initial search of the electronic databases, accessed on 18th July 2014, 1107 articles were found. Manuscript titles were reviewed for eligibility, and 1054 articles were excluded at this stage. 53 full papers were retrieved for more detailed evaluation. 29 studies met the full inclusion criteria, and all of these studies used 25(OH)D to determine the vitamin D concentration. Vitamin D herein refers to vitamin D_2 and D_3 (Figure 1). Whilst some studies measured only endothelial (6) or inflammatory parameters (14), the remaining studies measured both parameters (9) (Table 1).

3.1 Studies in type 2 and gestational diabetes mellitus/prediabetic participants

Whilst five studies investigated the effect of vitamin D supplementation in type 2 diabetes mellitus (T2DM) patients [15-18], two studies were performed in prediabetic participants [19] and pregnant women with gestational diabetes (GDM) [20] respectively. Of these studies, one [15], reported a significant increase of (mean ± SD) 2.35 ± 3.12 % in the endothelial function parameters, flow mediated dilation (FMD) from baseline (6.38 ± 4.31 %, $P = 0.048$) (Table1).

3.2 Studies in participants with a history of CVD

Seven studies recruited participants in this category [21-27] of which two reported significant improvement in FMD and CRP respectively. In one study, FMD was significantly better in the vitamin D group at 8 weeks compared to the placebo group (mean ± SD) 6.9 ± 3.5 % vs 3.7 ± 3.1 %, $P = 0.007$), but not at 16 weeks [23]. The second study reported a significant decrease in C-reactive protein (CRP, mean ±SD –vitamin D: 1.3 ± 5.4 mg/L vs placebo: +2.0 ± 6.8 mg/L, $P = 0.03$) [25] (Table 1).

3.3 Studies in participants with CKD

Three studies [28, 29, and 30] were conducted in patients with CKD. The study by Alvarez and colleagues [29] reported a significant decrease in monocyte chemo attractant protein-1 (MCP-1, mean ± SD) 66.2 ± 2.5 pg/mL – 60.8 ± 2.6 pg/mL, $P = 0.02$) at 12 weeks but not at 52 weeks, whereas the latter study did not observe a significant improvement in the endothelial/inflammatory markers measured (Table 1).

3.4 Studies in overweight/obese participants

Two studies recruited overweight participants and both reported a significant improvement in the inflammatory marker TNF-α (mean ± SD) 7.84 ± 3.15 pg/mL - 7.04 ± 2.25pg/mL, $P = 0.049$) [31], and FMD (mean ± SD) 54 ± 6s - 43 ± 3s, $P = 0.02$) [32] respectively. Another study [33] in obese participants, did not find significant improvement in the inflammatory markers measured (Table 1).

3.5 Studies in healthy participants

Five studies [34-38] recruited healthy participants, amongst these studies, one reported a significant improvement in the inflammatory marker hs-CRP (mean ± SD) 4.53 ± 0.64 µg/ml – 3.12 ± 0.46 µg/ml, $P = 0.01$) [34]. The remaining four studies did not report significant improvements in the markers/parameters measured.

3.6 Studies in participants with other conditions

One study recruited healthy post-menopausal women [39], and did not report significant improvements in both endothelial/inflammatory markers measured (Table 1). Four studies [40- 43], recruited participants with other diseases such as cystic fibrosis, human immunodeficiency virus (HIV), polycystic ovary syndrome and non-alcoholic fatty liver disease. Amongst these studies, one study [41], performed in cystic fibrosis patients, found significant reduction in an inflammatory marker, TNF- α (mean ± SD) 55.67 ± 8.18 pg/ml - 27.62 ± 5.82 pg/ml, $P < 0.01$) (Table 1).

Table 1. Characteristics of outcome of selected studies

Study, year, Country.	Participants /sample size /sex	Age of participants (mean ± SD)	Baseline 25(OH)D concentration (nmol/l) (mean ± SD)	Post – intervention 25(OH)D concentration (nmol/l) (mean ± SD)	Duration (weeks)	Type and dose of Vitamin D	Endothelial/ inflammatory parameters measured	Jadad score	Effect of intervention on outcome measures (mean ±SD)
Sugden *et al*., 2008 [15]. UK	T2DM patients n =34 Sex = M + F	64.9 ± 10.3 (Vitamin D group) 63.5 ± 9.5 (Placebo group)	40.2 ± 10.3 (Vitamin D group) 36.4 ± 8.5 (Placebo group)	63.1 ± 26.9 (Vitamin D group) 44 ± 21 (Placebo group)	8	100,000 IU single large dose of vitamin D_2 or placebo	FMD	3	Significant increase of 2.35 ± 3.12 % in FMD from baseline of 6.38 ± 4.31% (P = 0.048).
Zittermann *et al*., 2009 [31]. Germany	Overweight/ Obese subjects n = 165 Sex = M + F	47.4 ± 10.3 (Vitamin D group) 48.8 ± 10.1 (Placebo group)	30.0 ± 17.5 (Vitamin D group) 30.3 ± 20.1 (Placebo group)	85.5 ± 57.5 (Vitamin D group) 42.0 ± 35.0 (Placebo group)	52	3,332 IU vitamin D_3 or placebo daily	TNF-α, IL-6	5	Significant decrease in TNF-α (7.84 ± 3.15 pg/mL - 7.04 ± 2.25 pg/mL, P = 0.049), but not IL-6 (P > 0.05).
Witham *et al*., 2010 [16]. UK	T2DM patients n = 58 Sex = M + F	65.3 ± 11.1 100,000 IU 63.3 ± 9.6 200,000 IU (Vitamin D groups) 66.7 ± 9.7 (Placebo group)	41 ± 14 (100,000 IU) 48 ± 21 (200,000 IU) (Vitamin D groups) 45 ± 17 (Placebo group)	**8 weeks** 63 ± 20 (100000 IU) 79 ± 31 (200000 IU) (Vitamin D groups) 54 ± 20 (Placebo group) **16 weeks** 59 ± 18 (100000 IU) 76 ± 30 (200000 IU)	16	Single large dose of 100,000 or 200,000 IU of vitamin D_3 or placebo	FMD	5	No change in FMD at both 100,000 IU and 200,000 IU (P > 0.05).

				(Vitamin D groups) 53 ± 20 (Placebo group)					
Harris *et al*., 2011 [32]. USA	Healthy overweight African Americans n = 45 Sex = M+ F	29 ± 2 (Vitamin D group) 31 ± 2 (Placebo group)	34.3 ± 2.2 (Vitamin D group) 38.2 ± 3.0 (Placebo group)	100.9 ± 6.6 (Vitamin D group) 48.4 ± 3.2 (Placebo group)	16	60,000 IU of vitamin D_3 or placebo every four weeks	FMD	4	Significant improvement in FMD (54 ± 6s - 43 ± 3s, P = 0.02).
Barnes *et al*., 2011 [36]. UK	Healthy young and older patients n = 413 211 (aged 20 – 40y); 202 (aged ≥ 64y) Sex = M+F	30.5 ± 6.5 (200IU) 30.6 ± 5.5 (400 IU) 29.2 ± 6.8 (600 IU) (20-40 years) 70.7 ± 6.1 (200 IU); 70.6 ± 5.4 (400 IU) 71.2 ± 4.4 (600 IU) (≥ 64 years) (Vitamin D	**20-40 years** 60.1 (50.0,91.5) (200 IU); 72.2 (53.2- 93.4) (400 IU) 75.9 (55.4 - 89.4) (600 IU) (Vitamin D groups) 66.1 (57.2,95.5) (Placebo group) **≥ 64 years** 51.8 (40.3 -71.3) (200 IU); 55.5 (43.0 -72.3) (400 IU)	**20-40 years** 50.4 (45.0 – 60.4) (200 IU); 59.6 (51.3 – 70.3) (400 IU) 69.0 (59.1 -84.4) (600 IU); (Vitamin D groups) 38.9 (30.9,48.1) (Placebo group) **≥ 64 years** 42.6 (27.8 – 55.9) (200 IU) 70.3 (58.0-81.8) (400 IU);	22	200, 400 or 600 IU vitamin D_3 or placebo	hs-CRP, IL-6, IL-10 &TNF-α	5	No effect on the inflammatory markers measured (P > 0.05).

		groups) 29.9 ± 6.5 (Placebo group)	55.1 (39.4 – 70.8) (600 IU) (Vitamin D groups) 59.1(43.4,78.6) (Placebo group)	73.9 (61.9 – 90.2) (600 IU) (Vitamin D groups) 42.6 (27.8,55.9) (Placebo group)					
Stricker *et al*., 2012 [21]. Switzerland	Elderly PAD patients n = 62 Sex = M+ F	72.9 ± 8.7 (Vitamin D group) 74.8 ± 14.6 (Placebo group)	40.8 ± 16.8 (Vitamin D group) 42.5 ± 13.8 (Placebo group)	60.8 ± 15.5 (Vitamin D group) 43.8 ± 13.8 (Placebo group)	4	Single large dose of 100,000 IU vitamin D_2 or placebo	Skin blood flow, hs-CRP, D-dimer	4	No significant change in both endothelial and inflammatory markers ($P > 0.05$)
Witham *et al*., 2012 [23]. UK	Stroke patients n = 55 Sex = M+F	66.2 ± 13.0 (Vitamin D group) 67.7 ± 6.9 (Placebo group)	38.7 ± 17.6 (Vitamin D group) 37.8 ± 17.8 (Placebo group)	54 ± 15 (8 weeks) 51 ± 22 (16 weeks) (Vitamin D group) 42 ± 21 (8 weeks) 40 ± 19 (16 weeks) (Placebo group)	16	Single large dose of 100,000IU vitamin D_2 or placebo	FMD	3	FMD significantly better in vitamin D group at 8 weeks compared to placebo group (6.9 ± 3.5% vs 3.7 ± 3.1 %, $P = 0.007$), but did not improve at 16 weeks post intervention ($P > 0.05$).
Longenecker *et al*., 2012 [40]. USA	HIV Patients n = 44 Sex = M+F	47.0 ± 8.0 (Vitamin D group) 40 ± 10 (Placebo group)	Median (range): 22.5 (17.8 - 32.8) (Vitamin D group) 15.5 (9.3-24.5) (Placebo group)	Median (range): 35 (-15.6 – 51.3) (Vitamin D group) 11 (2 - 24.8) (Placebo group)	12	4,000 IU vitamin D_3 or placebo daily	FMD, CRP, IL-6, sTNF-1&2, sVCAM-1, sICAM-1,	5	No change in endothelial and inflammatory parameters measured ($P > 0.05$).

Sokol *et al*., 2012 [22]. USA	CAD patients n = 90 Sex = M+F	55 ± 9.6 (Vitamin D group) 56.9 ± 11.6 (Placebo group)	32.5 ± 17.5 (Vitamin D group) 35 ± 17.5 (Placebo group)	100 ± 45 (Vitamin D group) 37.5 ± 25 (Placebo group)	12	50,000 IU vitamin D_2 or placebo weekly	RH-PAT, FRHI, E-selectin hs - CRP, IL-6, IL-12, CXCL-10, and IFN-*y*	3	No change in inflammatory and endothelial parameters measured ($P > 0.05$)
Marckmann *et al*., 2012 [30] Denmark	Haemodialysis (HD) and non-haemodialysis CKD patients n = 52 Sex = M+F	Median (range): 71 (62-78) (Vitamin D group) 68 (59-76) (Placebo group)	**HD** Median (range): 20.7 (16.3 - 28.9) (Vitamin D group) 35.9 (25.5-45.9) (Placebo group) **Non-HD** 39.3 (17.6 - 50.2) (Vitamin D group) 28.6 (19.4 - 37.8) (Placebo group)	**HD** Median (range): 114.9 (82.5 - 153) (Vitamin D group) -10.4 (-21.4 to -6.5) (Placebo group) **Non-HD** 127.4 (104.9 - 155.2) (Vitamin D group) -7.1 (-12.3 to 9) (Placebo group)	8	40,000 IU vitamin D_3 or placebo weekly	vWF, IL-6, aPWV, D-dimer and CRP	4	No change in endothelial and inflammatory markers measured ($P > 0.05$)
Grossmann *et al*., 2012 [41] USA	Cystic fibrosis patients n =30 Sex =M+F	24.9 ± 16.0 (Vitamin D group) 28.2 ± 30.9 (Placebo group)	76.5 ± 8.0 (Vitamin D group) 71.7 ± 8.8 (Placebo group)	91.8 ± 6.5 (Vitamin D group) 70 ± 10.3 (Placebo group)	12	Large bolus dose of 250, 000 IU vitamin D_3 or placebo	TNF-α, IL-6, IL-8, IL-10, IL-1β, IL-18BP and NGAL	5	Significant decrease in TNF-α (55.67 ± 8.18 pg/ml - 27.62 ± 5.82 pg/ml, $P < 0.01$), but not in IL-6,IL-1β, IL-8, IL-10,IL-18BP and NGAL ($P > 0.05$).

Witham *et al.*, 2013 [38]. UK	South Asian women living in UK n = 50 Sex = F	41.7 ± 13.4 (Vitamin D group) 39.4 ± 11.8 (Placebo group)	27 ± 13 (Vitamin D group) 27 ± 15 (Placebo group)	43 ± 13.0 (4 weeks) 38 ± 15.0 (8 weeks) (Vitamin D group) 27 ± 17 (Placebo group)	8	Single large dose of 100, 000 IU vitamin D_3 or placebo	FMD, TNF-α, IL-6, E-selectin	5	No change in endothelial and inflammatory markers at 4 and 8 weeks ($P > 0.05$)
Witham *et al*. 2013 [25]. UK	Patients with history of MI n = 74 Sex = M + F	64.3 ± 9.8 (Vitamin D group) 67.5 ± 10.6 (Placebo group)	49.0 ± 20.0 (Vitamin D group) 45 ± 16 (Placebo group)	56 ± 20 (8 weeks) 62 ± 20 (26 weeks) (Vitamin D group) 45 ± 16 (8 weeks) 46 ± 16 (16 weeks) (Placebo group)	26	Single large dose 100,000 IU vitamin D_3 or placebo at baseline, 8 and 26 weeks.	TNFα, CRP, vWF, RHI and E-selectin	5	Significant decrease in CRP – 1.3 ± 5.4mg/L (vitamin D group) vs +2 ± 6.8 mg/L (placebo group), $P = 0.03$), but no improvement TNF-α, vWF, RHI, and E-selectin ($P > 0.05$)
Brevlasky *et al*. 2013 [17]. Israel	T2DM patients, n = 47 Sex = M+F	66.8 ± 9.2 (Vitamin D group) 65.8 ± 9.7 (Placebo group)	29.5 ± 27.3 (Vitamin D group) 32.3 ± 26.7 (Placebo group)	44 ± 28.8 (Vitamin D group) 35 ± 14.8 (Placebo group)	52	1,000 IU vitamin D_3 or placebo daily	hs-CRP	3	No change in hs-CRP ($P > 0.05$)
Alvarez *et al*. 2013 [29]. USA	Early CKD (stage 2-3) patients n = 47 Sex = M+F	62.5 ± 9.6	67.5 ± 17.5 (Vitamin D group) 80 ± 22.5 (Placebo group)	77 ± 122 (12 weeks) 73 ±114 (52 weeks) (Vitamin D group) -18 ± 19 (12 weeks) - 5 ± 19 (52 weeks) (Placebo group)	52	50,000 IU vitamin D_3 or placebo 12 weeks followed by 50,000 IU	TNF-α, IL-6, MCP-1	3	Significant decrease in MCP-1 at 12 weeks (66.2 ± 2.5pg/mL – 60.8 ± 2.6pg/mL, $P = 0.02$), but not at 52 weeks. No change in the other inflammatory markers

						every other week for 40 weeks			measured ($P > 0.05$)
Wood *et al*. 2012 [38]. UK	Post-menopausal women n = 265 Sex = F	63.5 ± 1.9 (400 IU) 64.1 ± 2.3 (1000 IU) (Vitamin D group) 63.9 ± 2.3 (Placebo group)	33.3 ± 13.2 (400IU) 33.4 ±13.9 (1000 IU) (Vitamin D groups) 36.3 ± 16.4 (Placebo group)	64.9 ± 19.8 (400 IU) 75.7 ± 19.1(1000 IU) (Vitamin D groups) 32.4 ± 14.7 (Placebo group)	52	400 or 1,000 IU vitamin D_3 or placebo daily	hs-CRP, IL-6, ICAM-1	5	No change in inflammatory markers measured ($P > 0.05$).
Yiu *et al*. 2013 [18]. Hong Kong	T2DM patients n = 100 Sex = M	65.8 ± 7.3 (Vitamin D group) 64.9 ± 8.9 (Placebo group)	52.8 ± 11.0 (Vitamin D group) 54.8 ± 10.3 (Placebo group)	146.5 (mean) (Vitamin D group) 59.5 (mean) (Placebo group)	12	5,000 IU vitamin D_3 or placebo daily	FMD, EPC, PWV & hs-CRP	3	No change in both endothelial and inflammatory markers measured ($P > 0.05$)
Witham et al. 2013 [24] UK	Older patients with isolated systolic hypertension n =159 Sex = M	76.9 ± 4.8 (Vitamin D group) 76.7 ± 4.5 (Placebo group)	45 ± 15 (Vitamin D group) 45 ± 15 (Placebo group)	69 ±23 (26 weeks) 67 ±17 at (52 weeks) (Vitamin D group) 52 ±22 at (26 weeks) 48 ±18 at (52 weeks) (Placebo group)	52	100,000 IU vitamin D_3 or placebo every 3 months	FMD	5	No change in FMD after intervention ($P > 0.05$)

Rahimi-Ardabili *et al*. 2013 [42] Iran	Polycystic ovary syndrome patients n = 50 Sex = F	26.8 ± 4.7 (Vitamin D group) 27.0 ± 3.7 (Placebo group)	17.3 ± 7 (Vitamin D group) 18.2 ± 7.3 (Placebo group)	58.5 ± 15.4 (Vitamin D group) 21.4 ± 9.9 (Placebo group)	8	One 50,000IU vitamin D_3 or placebo capsules every 20 days	hs-CRP	5	No change in hs-CRP ($P > 0.05$)
Sollid *et al*. 2014 [19] Norway	Patients with pre-diabetes n = 484 sex = M+F	62.3 ± 8.1 (Vitamin D group) 61.9 ± 9.2 (Placebo group)	59.9 ± 21.9 (Vitamin D group) 61.1 ± 21.2 (Placebo group)	105.7 ± 46.1 (Vitamin D group) 64.5 ± 38.1 (Placebo group)	52	20,000 IU vitamin D_3 or placebo capsules weekly	hs- CRP	5	No change in hs-CRP ($P > 0.05$)
Yusupov *et al*. 2010 [37] USA	Ambulatory adults n = 120 Sex	59.3 ± 13.0 (Vitamin D group) 58.1 ±13.9 (Placebo group)	64.3 ± 25.4 (Vitamin D group) 63 ± 25.8 (Placebo group)	88.5 ± 23.2 (Vitamin D group) 95.7 ± 46.1 (Placebo group)	13	2,000 IU vitamin D_3 or placebo daily	IL - 2,4,5,6,8,10,13, GM-CSF, IFN-*y*, TNF-α	5	No change in cytokines measured ($P > 0.05$)
Sharifi *et al*. 2014 [43] Iran	Patients with non-alcoholic fatty liver disease n = 53 Sex = M+F	40.3 ± 8.7 (Vitamin D group) 43.9 ± 9.5 (Placebo group)	Median (range): 28.8 (22.0-71.0) (Vitamin D group) 42.1(29.8-62) (Placebo group)	Median (range): 75 (64.5- 116.5) (Vitamin D group) 48 (36.8 – 66.8) (Placebo group)	17	50,000 IU vitamin D_3 or placebo every 14 days	hs-CRP, TNF-α	5	No change in inflammatory markers (hs- CRP and TNF-α) measured ($P > 0.05$).

Barker *et al*. 2012 [35] USA	Vitamin D sufficient adults n = 30 Sex = M+F	26.6 ± 3.2 (200 IU) 29 ±5 (4000 IU) (Vitamin D groups) 30.2 ± 4.8 (Placebo group)	36.3 ± 11.3 (200 IU) 32.5 ± 6.56 (4000 IU) (Vitamin D groups) 27.8 ± 10.7 (Placebo group)	37.5 ± 8.83 (200 IU) 50.1 ± 5.81 (4000 IU) (Vitamin D groups) 25.1 ± 9.34 (Placebo group)	4	200 or 4000 IU vitamin D_3 or placebo daily	IL-5, IL-10, IFN-γ	2	No change in inflammatory markers ($P > 0.05$)
Witham *et al*., 2010 [26] UK	Older patients with heart failure n = 96 Sex = M+F	78.8 ± 5.6 (Vitamin D group) 80.6 ± 5.7 (Placebo group)	20.5 ± 8.9 (Vitamin D group) 23.7 ± 10 (Placebo group)	43.4 ± 30.9 (10 weeks) 40 ± 22.8 (20 weeks) (Vitamin D group) 26 ± 20.9 (10 weeks) 25 ± 23.4 (20 weeks) (Placebo group)	20	100, 000 IU vitamin D_2 or placebo at baseline and 10 weeks	TNF-α,	5	No change in in TNF-α ($P > 0.05$)
Asemi *et al*., 2013 [34] Iran	Healthy pregnant women n = 48 Sex = F	25.3 ± 4.2 (Vitamin D group) 24.8 ± 3.6 (Placebo group)	44.5 ± 3.3 (Vitamin D group) 36.25 ± 3.0 (Placebo group)	53.8 ± 4.5 (Vitamin D group) 33.25 ± 2.75 (Placebo group)	9	400 IU vitamin D_3 or placebo daily	hs-CRP	5	Significant improvement in hs-CRP (4.53 ± 0.64 µg/ml – 3.12 ± 0.46 µg/ml, ($P = 0.01$)

Wamberg *et al*., 2013 [33] Denmark	Obese adults n = 52 Sex = M + F	39.5 ± 8.0 (Vitamin D group) 41.2 ± 6.8 (Placebo group)	33.0 ± 10.8 (Vitamin D group) 34 ± 9 (Placebo group)	110.2 ± 21.2 (Vitamin D group) 46.8 ± 17.3 (Placebo group)	26	7000 IU vitamin D_3 or placebo daily	hs-CRP, IL-6 and MCP-1	5	No change in markers measured ($P > 0.05$)
Hewitt *et al* 2013 [28] Australia	CKD-5D hemodialysis patients n = 60 Sex = M+F	Median (range): 60 (53-71) (Vitamin D group) Median (range): 67 (54-72) (Placebo group)	45 ± 12.5 (Vitamin D group) 40 ± 12.5 (Placebo group)	87.5 ± 22.5 (Vitamin D group) 40 ± 17.5 (Placebo group)	26	50,000 IU vitamin D_3 or placebo weekly for 8 weeks, followed by monthly doses for the remaining 18 weeks	PWV,CRP	4	No change in markers Measured ($P > 0.05$)

Abbreviations: AI, augmentation index; ARV, antiretroviral; BNP, B – type natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; CRP, C-reactive protein; CXCL-10, C-X-C motif chemokine 10; EFV, Efavirenz; EPCs, endothelial progenitor cells; FMD, flow mediated dilatation; FRHI, Framingham reactive hyperemia index; GDM, gestational diabetes mellitus; HD, haemodialysis; HIV, human immunodeficiency virus; hs- CRP, high sensitivity – C-reactive protein; IL, interleukin; IFN-γ, interferon gamma; IU, international units; MCP-1, monocyte chemo attractant protein-1; MI, myocardial infarction; NGAL, neutrophil gelatinase associated lipocalin; NR, not reported; PAD, peripheral artery disease; PWV, pulse wave velocity; RHI, reactive hyperaemia index; RCT, randomised controlled trial; RH-PAT, reactive hyperaemia peripheral arterial tonometry; Sicam-1, soluble intracellular molecule-1; TNF, tumour necrosis factor alpha; T2DM, type 2 diabetes mellitus; vWF, von Willebrand factor.

Discussion

To the best of our knowledge, this is the first systematic review aimed at evaluating the effect of supplemental vitamin D on endothelial function by examining inflammatory biomarkers/parameters in humans. Overall, 8 studies reported significant improvements in the biomarkers/parameters measured. However, in 2 out of the 8 studies, improvements were reported at interim time points; however, these improvements were absent post intervention. Reported improvements were mainly in FMD measurements, but there were no common biomarkers that showed an improvement in all the studies (Table 1).

Several mechanisms have been proposed that may account for the reported improvement in endothelial and inflammatory biomarkers following vitamin D supplementation. In 2013, Kassi *et al.* [44] suggested that a principal contributory factor to endothelial dysfunction is the diminished availability of NO, and the rise in the production of free radicals. Hence, the proposed direct mechanism by which vitamin D may improve endothelial function may be by enhancing nitric oxide production and decreasing the production of free radicals.

A study by Molinari *et al.*, [45], examined the effects of vitamin D on NO production and p38, Akt, ERK and eNOS phosphorylations in human umbilical vein cell culture (HUVEC). It was discovered that vitamin D, acting through its VDR and endothelial NO synthase activation, promotes a significant rise in NO production in the endothelium. Another proposed mechanism for reported improvement could be, the increase in plasma 25(OH)D concentration following vitamin D supplementation. This increase would in turn lead to an increase in the intestinal absorption of calcium, which may result in increased intracellular calcium, which would in turn stimulate the production of NO, a potent vasodilator purported to have protective effects on the endothelium. This is an indirect route by which vitamin D increases nitric oxide availability [46].

A further indirect mechanism by which vitamin D may account for the improved endothelial function/inflammatory markers observed in the aforementioned studies may be by the way of reducing blood pressure. This is achieved by downregulation of renin and angiotensinogen gene expression through a vitamin D response element in the promoter region of the renin gene by obstructing the NF-kB pathway [47]. One study, carried out in rodents, found that in wild-type mice, inhibition of 1,25-dihydroxyvitamin D_3 synthesis by

adding 2.5% strontium chloride in their normal diet led to an increase in renin expression, whereas the injection of vehicle or 30 pmol $1,25(OH)_2D_3$ dissolved in propylene glycol led to renin suppression, independent of calcium metabolism [48]. Some of the included study participants had T2DM and others were uremic patients. Hence, vitamin D acting through VDR may have improved endothelial dysfunction by hindering the damaging effects of advanced glycation end products (which have been reported to be increased in diabetic and uremic patients) by reducing the expression of genes such as IL-6, and IL-8 involved in the advanced glycation end products-activated inflammatory pathway [49].

The participants in one of the studies which recorded a significant improvement in TNF-α, were undergoing a weight reduction programme. The loss in weight following this programme may, at least in part, be responsible for this improvement, as it has been found that adipose tissue expresses TNF- α [50].

Most of the included study participants, had disease states which are characterised by inflammation. Thus, vitamin D may have played an anti-inflammatory role by repressing NF-kB activation by enhancing the gene expression of 1kBα in macrophages and peripheral blood mononuclear cells, thereby disrupting the movement of upregulated NF-kB subunit p65 to the nucleus [51].

The majority of studies included in this systematic review however, did not report significant improvement in endothelial function following intervention. It may be the case that vitamin D supplementation may in fact not elicit clinical/health effects, however, several explanations are possible for the lack of effect of vitamin D supplementation in many of the included studies.

One study recruited a population at risk of vitamin D deficiency such as post-menopausal women. At menopause there is a transition in vitamin D requirements, as the VDR depends on oestrogen which declines with age [52, 53]. As the number of VDR decreases following a reduction in oestrogen, this leads to alterations in the quantity and functionality of the VDR in target tissues [54]. Target cell response to vitamin D is dependent on VDR levels and factors such as glucocorticoids and retinoids as well as oestrogen, are known to modulate VDR levels [55]. This is supported by evidence from studies in animal models (rats), that shows oestrogen increases the expression of VDR in various tissues such as endothelial,

colon, immune and smooth muscle cells [56, 57]. It could therefore be assumed that at menopause, which is characterised by a reduction in oestrogen, there will be a decrease in VDR, which in turn may explain a lack of improvement in both endothelial and inflammatory biomarkers/ parameters measured. The use of a higher (e.g. 5,000 IU and above) daily dose in populations at risk of vitamin D deficiency and in individuals with 25(OH)D levels below 55nmol/l is recommended, as this has previously produced significant improvement in vitamin D status in participants with particularly low levels of 25(OH)D [58, 59].

Several included studies, recruited participants with established CVD who were taking statins and anti-hypertensive drugs. Vitamin D is a derivative of cholesterol, and statins lower serum cholesterol concentration by inhibiting HMG CoA reductase, the rate limiting enzyme in cholesterol synthesis. Therefore, by reducing cholesterol synthesis, statins also interfere with vitamin D metabolism [60, 61]. The interaction between vitamin D and the drugs taken by participants in these studies may also be significant as they may affect vitamin D metabolism by competing for CYP3A4 enzyme activity. Amongst the six cytochrome P450 enzymes that catalyse reactions involved in the metabolism of up to 90% of therapeutic drugs, CYP3A4 and CYP2D6 are most important [62]. CYP3A4 is a hepatic 25-hydroxylase enzyme that converts inactive to active vitamin D, and is also responsible for the metabolism of antihypertensive drugs along with some statins such as Atorvastatin, Lovastatin and Simvastatin. These drugs are CYP3A4 enzyme inhibitors, and therefore all compete for the same enzyme receptor site, with the more potent drug dominating. This results in a decreased metabolism of the competing drug which is vitamin D in this case, therefore reducing its efficacy. This could explain the slight increase in serum 25(OH)D despite the large vitamin D doses administered. The studies in which participants were taking statins didn't state the exact type of statins they were using, which is significant as only statins metabolised by CYP3A4 can interact with vitamin D [63-65].

Some participants in the included studies already had established and widespread CVD, this could have led to irreversible anatomical changes in the vascular wall, enough to prevent positive impact of the active treatment. Genetic variation (polymorphism) of CYP3A4 may influence an individual's response to vitamin D [66]. Old age is a contributing factor to individual variation in vitamin D metabolism and some of the included study participants were older adults. The cytochrome P450 monooxygenase system is more affected by aging

than by any other metabolic pathway due to a reduction in enzyme activity, hepatic blood flow and liver mass result in a decreased metabolic activity [67].

One study included in this review recruited HIV patients [40] who were undertaking stable antiretroviral therapy. There could be increased catabolism of vitamin D in this study, as increased intake of the antiretroviral drug Efavirenz (EFV), has been reported by Childs *et al*., [68] to reduce the expression of CYP2RI, an enzyme involved in the 25- hydroxylation of vitamin D_3, and also induce the expression of CYP24 which converts vitamin D into its inactive metabolite.

Limitations of the present systematic review are acknowledged in that only RCTs were included, which are known to provide more reliable data by reducing the possibility of confounding or selection bias. The included RCTs only supplemented with vitamin D, and excluded RCTs which supplemented with both calcium and vitamin D to allow adequate conclusions on the effect of vitamin D to be drawn. However, it is acknowledged that there is also some heterogeneity between studies, as study populations were diverse, with different diseases, follow-up duration and vitamin D doses; the majority of which were administered as single large, monthly and weekly doses. The vitamin D doses administered in some of the studies included were insufficient to cause an overall change in the inflammatory/endothelial markers measured. Some studies used healthy participants and therefore the elevated levels of inflammatory markers that were seen in participants with CVD were absent.

In conclusion, this systematic review did not on the whole, demonstrate that vitamin D supplementation in adults leads to improved circulating inflammatory and endothelial function biomarkers/parameters. It can therefore be concluded that based upon this systematic review, a strong rationale does not yet exist for the therapeutic administration of supplemental vitamin D in order to attenuate CVD risk. It is also possible that the anti–inflammatory role of vitamin D may not occur at the systemic level but instead occur at the cellular level. Furthermore, many studies in relation to vitamin D, have used CVD risk as a secondary outcome and therefore may not be sufficiently powered in relation to CVD endpoints.

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References

[1]. Vanchinathan V, Lim HW. A dermatologist's perspective on vitamin D. Mayo Clinic proceedings. 2012;87(4):372-80.

[2]. Lee JH, O'Keefe JH, Bell D, Hensrud DD, Holick MF. Vitamin D deficiency an important, common, and easily treatable cardiovascular risk factor? J Am Coll Cardiol. 2008;52(24):1949-56.

[3]. Pfeifer M, Begerow B, Minne HW. Vitamin D and muscle function. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2002;13(3):187-94.

[4]. Heaney RP. Vitamin D in health and disease. Clinical journal of the American Society of Nephrology : CJASN. 2008;3(5):1535-41.

[5]. Cohen-Lahav M, Douvdevani A, Chaimovitz C, Shany S. The anti-inflammatory activity of 1,25-dihydroxyvitamin D3 in macrophages. The Journal of steroid biochemistry and molecular biology. 2007;103(3-5):558-62.

[6]. Zittermann A. Vitamin D and disease prevention with special reference to cardiovascular disease. Progress in biophysics and molecular biology. 2006;92(1):39-48.

[7]. Geleijnse JM. Vitamin D and the prevention of hypertension and cardiovascular diseases: a review of the current evidence. American journal of hypertension. 2011;24(3):253-62.

[8]. Giovannucci E, Liu Y, Hollis BW, Rimm EB. 25-hydroxyvitamin D and risk of myocardial infarction in men: a prospective study. Arch Intern Med. 2008;168(11):1174-80.

[9]. Chitalia N, Ismail T, Tooth L, Boa F, Hampson G, Goldsmith D, et al. Impact of vitamin D supplementation on arterial vasomotion, stiffness and endothelial biomarkers in chronic kidney disease patients. PloS one. 2014;9(3):e91363.

[10]. Schleithoff SS, Zittermann A, Tenderich G, Berthold HK, Stehle P, Koerfer R. Vitamin D supplementation improves cytokine profiles in patients with congestive heart failure: a double-blind, randomized, placebo-controlled trial. Am J Clin Nutr. 2006;83(4):754-9.

[11]. McGorisk GM, Treasure CB. Endothelial dysfunction in coronary heart disease. Current opinion in cardiology. 1996;11(4):341-50.

[12]. Munzel T, Sinning C, Post F, Warnholtz A, Schulz E. Pathophysiology, diagnosis and prognostic implications of endothelial dysfunction. Annals of medicine. 2008;40(3):180-96.

[13]. Romagnoli E, Mascia ML, Cipriani C, Fassino V, Mazzei F, D'Erasmo E, et al. Short and long-term variations in serum calciotropic hormones after a single very large dose of ergocalciferol (vitamin D2) or cholecalciferol (vitamin D3) in the elderly. The Journal of clinical endocrinology and metabolism. 2008;93(8):3015-20.

[14]. Jadad AR, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, et al. Assessing the quality of reports of randomized clinical trials: is blinding necessary? Controlled clinical trials. 1996;17(1):1-12.

[15]. Sugden JA, Davies JI, Witham MD, Morris AD, Struthers AD. Vitamin D improves endothelial function in patients with Type 2 diabetes mellitus and low vitamin D levels. Diabetic medicine : a journal of the British Diabetic Association. 2008;25(3):320-5.

[16]. Witham MD, Dove FJ, Dryburgh M, Sugden JA, Morris AD, Struthers AD. The effect of different doses of vitamin D(3) on markers of vascular health in patients with type 2 diabetes: a randomised controlled trial. Diabetologia. 2010;53(10):2112-9.

[17]. Breslavsky A, Frand J, Matas Z, Boaz M, Barnea Z, Shargorodsky M. Effect of high doses of vitamin D on arterial properties, adiponectin, leptin and glucose homeostasis in type 2 diabetic patients. Clinical nutrition. 2013;32(6):970-5.

[18]. Yiu YF, Yiu KH, Siu CW, Chan YH, Li SW, Wong LY, et al. Randomized controlled trial of vitamin D supplement on endothelial function in patients with type 2 diabetes. Atherosclerosis. 2013;227(1):140-6.

[19]. Sollid ST, Hutchinson MY, Fuskevag OM, Figenschau Y, Joakimsen RM, Schirmer H, et al. No effect of high-dose vitamin D supplementation on glycemic status or cardiovascular risk factors in subjects with prediabetes. Diabetes care. 2014;37(8):2123-31.

[20]. Asemi Z, Hashemi T, Karamali M, Samimi M, Esmaillzadeh A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. Am J Clin Nutr. 2013;98(6):1425-32.

[21]. Stricker H, Tosi Bianda F, Guidicelli-Nicolosi S, Limoni C, Colucci G. Effect of a single, oral, high-dose vitamin D supplementation on endothelial function in patients with peripheral arterial disease: a randomised controlled pilot study. European journal of vascular and endovascular surgery : the official journal of the European Society for Vascular Surgery. 2012;44(3):307-12.

[22]. Sokol SI, Srinivas V, Crandall JP, Kim M, Tellides G, Lebastchi AH, et al. The effects of vitamin D repletion on endothelial function and inflammation in patients with coronary artery disease. Vascular medicine. 2012;17(6):394-404.
[23]. Witham MD, Dove FJ, Sugden JA, Doney AS, Struthers AD. The effect of vitamin D replacement on markers of vascular health in stroke patients - a randomised controlled trial. Nutrition, metabolism, and cardiovascular diseases : NMCD. 2012;22(10):864-70.
[24]. Witham MD, Price RJ, Struthers AD, Donnan PT, Messow CM, Ford I, et al. Cholecalciferol treatment to reduce blood pressure in older patients with isolated systolic hypertension: the VitDISH randomized controlled trial. JAMA internal medicine. 2013;173(18):1672-9.
[25]. Witham MD, Dove FJ, Khan F, Lang CC, Belch JJ, Struthers AD. Effects of vitamin D supplementation on markers of vascular function after myocardial infarction--a randomised controlled trial. International journal of cardiology. 2013;167(3):745-9.
[26]. Witham MD, Crighton LJ, Gillespie ND, Struthers AD, McMurdo ME. The effects of vitamin D supplementation on physical function and quality of life in older patients with heart failure: a randomized controlled trial. Circulation Heart failure. 2010;3(2):195-201.
[27]. Larsen T, Mose FH, Bech JN, Hansen AB, Pedersen EB. Effect of cholecalciferol supplementation during winter months in patients with hypertension: a randomized, placebo-controlled trial. American journal of hypertension. 2012;25(11):1215-22.
[28]. Hewitt NA, O'Connor AA, O'Shaughnessy DV, Elder GJ. Effects of cholecalciferol on functional, biochemical, vascular, and quality of life outcomes in hemodialysis patients. Clinical journal of the American Society of Nephrology : CJASN. 2013;8(7):1143-9.
[29]. Alvarez JA, Zughaier SM, Law J, Hao L, Wasse H, Ziegler TR, et al. Effects of high-dose cholecalciferol on serum markers of inflammation and immunity in patients with early chronic kidney disease. European journal of clinical nutrition. 2013;67(3):264-9.
[30]. Marckmann P, Agerskov H, Thineshkumar S, Bladbjerg EM, Sidelmann JJ, Jespersen J, et al. Randomized controlled trial of cholecalciferol supplementation in chronic kidney disease patients with hypovitaminosis D. Nephrology, dialysis, transplantation : official publication of the European Dialysis and Transplant Association - European Renal Association. 2012;27(9):3523-31.
[31]. Zittermann A, Frisch S, Berthold HK, Gotting C, Kuhn J, Kleesiek K, et al. Vitamin D supplementation enhances the beneficial effects of weight loss on cardiovascular disease risk markers. Am J Clin Nutr. 2009;89(5):1321-7.

[32]. Harris RA, Pedersen-White J, Guo DH, Stallmann-Jorgensen IS, Keeton D, Huang Y, et al. Vitamin D3 supplementation for 16 weeks improves flow-mediated dilation in overweight African-American adults. American journal of hypertension. 2011;24(5):557-62.
[33]. Wamberg L, Kampmann U, Stodkilde-Jorgensen H, Rejnmark L, Pedersen SB, Richelsen B. Effects of vitamin D supplementation on body fat accumulation, inflammation, and metabolic risk factors in obese adults with low vitamin D levels - results from a randomized trial. European journal of internal medicine. 2013;24(7):644-9.
[34]. Asemi Z, Samimi M, Tabassi Z, Shakeri H, Esmaillzadeh A. Vitamin D supplementation affects serum high-sensitivity C-reactive protein, insulin resistance, and biomarkers of oxidative stress in pregnant women. The Journal of nutrition. 2013;143(9):1432-8.
[35]. Barker T, Martins TB, Hill HR, Kjeldsberg CR, Henriksen VT, Dixon BM, et al. Different doses of supplemental vitamin D maintain interleukin-5 without altering skeletal muscle strength: a randomized, double-blind, placebo-controlled study in vitamin D sufficient adults. Nutrition & metabolism. 2012;9(1):16.
[36]. Barnes MS, Horigan G, Cashman KD, Hill TR, Forsythe LK, Lucey AJ, et al. Maintenance of wintertime vitamin D status with cholecalciferol supplementation is not associated with alterations in serum cytokine concentrations among apparently healthy younger or older adults. The Journal of nutrition. 2011;141(3):476-81.
[37]. Yusupov E, Li-Ng M, Pollack S, Yeh JK, Mikhail M, Aloia JF. Vitamin d and serum cytokines in a randomized clinical trial. International journal of endocrinology. 2010;2010.
[38]. Witham MD, Adams F, Kabir G, Kennedy G, Belch JJ, Khan F. Effect of short-term vitamin D supplementation on markers of vascular health in South Asian women living in the UK--a randomised controlled trial. Atherosclerosis. 2013;230(2):293-9.
[39]. Wood AD, Secombes KR, Thies F, Aucott L, Black AJ, Mavroeidi A, et al. Vitamin D3 supplementation has no effect on conventional cardiovascular risk factors: a parallel-group, double-blind, placebo-controlled RCT. The Journal of clinical endocrinology and metabolism. 2012;97(10):3557-68.
[40]. Longenecker CT, Hileman CO, Carman TL, Ross AC, Seydafkan S, Brown TT, et al. Vitamin D supplementation and endothelial function in vitamin D deficient HIV-infected patients: a randomized placebo-controlled trial. Antiviral therapy. 2012;17(4):613-21.
[41]. Grossmann RE, Zughaier SM, Liu S, Lyles RH, Tangpricha V. Impact of vitamin D supplementation on markers of inflammation in adults with cystic fibrosis hospitalized for a pulmonary exacerbation. European journal of clinical nutrition. 2012;66(9):1072-4.

[42]. Rahimi-Ardabili H, Pourghassem Gargari B, Farzadi L. Effects of vitamin D on cardiovascular disease risk factors in polycystic ovary syndrome women with vitamin D deficiency. Journal of endocrinological investigation. 2013;36(1):28-32.

[43]. Sharifi N, Amani R, Hajiani E, Cheraghian B. Does vitamin D improve liver enzymes, oxidative stress, and inflammatory biomarkers in adults with non-alcoholic fatty liver disease? A randomized clinical trial. Endocr. 2014.

[44]. Kassi E, Adamopoulos C, Basdra EK, Papavassiliou AG. Role of vitamin D in atherosclerosis. Circulation. 2013;128(23):2517-31.

[45]. Molinari C, Uberti F, Grossini E, Vacca G, Carda S, Invernizzi M, et al. 1alpha,25-dihydroxycholecalciferol induces nitric oxide production in cultured endothelial cells. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology. 2011;27(6):661-8.

[46]. Schneider JC, El Kebir D, Chereau C, Lanone S, Huang XL, De Buys Roessingh AS, et al. Involvement of Ca2+/calmodulin-dependent protein kinase II in endothelial NO production and endothelium-dependent relaxation. American journal of physiology Heart and circulatory physiology. 2003;284(6):H2311-9.

[47]. Deb DK, Chen Y, Zhang Z, Zhang Y, Szeto FL, Wong KE, et al. 1,25-Dihydroxyvitamin D3 suppresses high glucose-induced angiotensinogen expression in kidney cells by blocking the NF-{kappa}B pathway. Am J Physiol Renal Physiol. 2009;296(5):F1212-8.

[48]. Li YC, Kong J, Wei M, Chen ZF, Liu SQ, Cao LP. 1,25-Dihydroxyvitamin D(3) is a negative endocrine regulator of the renin-angiotensin system. J Clin Invest. 2002;110(2):229-38.

[49]. Talmor Y, Golan E, Benchetrit S, Bernheim J, Klein O, Green J, et al. Calcitriol blunts the deleterious impact of advanced glycation end products on endothelial cells. Am J Physiol Renal Physiol. 2008;294(5):F1059-64.

[50]. Kern PA, Ranganathan S, Li C, Wood L, Ranganathan G. Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. Am J Physiol Endocrinol Metab. 2001;280(5):E745-51.

[51]. Krishnan AV, Feldman D. Mechanisms of the anti-cancer and anti-inflammatory actions of vitamin D. Annual review of pharmacology and toxicology. 2011;51:311-36.

[52]. Compston JE. Sex steroids and bone. Physiological reviews. 2001;81(1):419-47.

[53]. Duque G, El Abdaimi K, Macoritto M, Miller MM, Kremer R. Estrogens (E2) regulate expression and response of 1,25-dihydroxyvitamin D3 receptors in bone cells:

changes with aging and hormone deprivation. Biochemical and biophysical research communications. 2002;299(3):446-54.

[54]. Horst RL, Goff JP, Reinhardt TA. Advancing age results in reduction of intestinal and bone 1,25-dihydroxyvitamin D receptor. Endocrinology. 1990;126(2):1053-7.

[55]. Bortman P, Folgueira MA, Katayama ML, Snitcovsky IM, Brentani MM. Antiproliferative effects of 1,25-dihydroxyvitamin D3 on breast cells: a mini review. Brazilian journal of medical and biological research = Revista brasileira de pesquisas medicas e biologicas / Sociedade Brasileira de Biofisica [et al]. 2002;35(1):1-9.

[56]. Smirnoff P, Liel Y, Gnainsky J, Shany S, Schwartz B. The protective effect of estrogen against chemically induced murine colon carcinogenesis is associated with decreased CpG island methylation and increased mRNA and protein expression of the colonic vitamin D receptor. Oncology research. 1999;11(6):255-64.

[57]. Zhou Y, Ye RQ, Cai DH, Zhang H. Effect of estrogen and progesterone on the expression of 1, 25-dihydroxyvitamin D receptors mRNA in the liver of ovariectomized rats. Di 1 jun yi da xue xue bao = Academic journal of the first medical college of PLA. 2002;22(6):521-3.

[58]. Aloia JF, Patel M, Dimaano R, Li-Ng M, Talwar SA, Mikhail M, et al. Vitamin D intake to attain a desired serum 25-hydroxyvitamin D concentration. Am J Clin Nutr. 2008;87(6):1952-8.

[59]. Chel V, Wijnhoven HA, Smit JH, Ooms M, Lips P. Efficacy of different doses and time intervals of oral vitamin D supplementation with or without calcium in elderly nursing home residents. Osteoporosis international : a journal established as result of cooperation between the European Foundation for Osteoporosis and the National Osteoporosis Foundation of the USA. 2008;19(5):663-71.

[60]. Istvan ES, Deisenhofer J. Structural mechanism for statin inhibition of HMG-CoA reductase. Science. 2001;292(5519):1160-4.

[61]. Montagnani M, Lore F, Di Cairano G, Gonnelli S, Ciuoli C, Montagnani A, et al. Effects of pravastatin treatment on vitamin D metabolites. Clinical therapeutics. 1994;16(5):824-9.

[62]. Lynch T, Price A. The effect of cytochrome P450 metabolism on drug response, interactions, and adverse effects. American family physician. 2007;76(3):391-6.

[63]. Williams D, Feely J. Pharmacokinetic-pharmacodynamic drug interactions with HMG-CoA reductase inhibitors. Clinical pharmacokinetics. 2002;41(5):343-70.

[64]. Vaughan CJ, Gotto AM. Update on statins: 2003. Circulation. 2004;110(7):886-92.

[65]. Zhou S-F. Drugs behave as substrates, inhibitors and inducers of human cytochrome P450 3A4. Current drug metabolism. 2008;9(4):310-22.

[66]. Wilkinson GR. Drug metabolism and variability among patients in drug response. The New England journal of medicine. 2005;352(21):2211-21.

[67]. Weinshilboum R. Inheritance and drug response. The New England journal of medicine. 2003;348(6):529-37.

[68]. Childs K, Welz T, Samarawickrama A, Post FA. Effects of vitamin D deficiency and combination antiretroviral therapy on bone in HIV-positive patients. Aids. 2012;26(3):253-62.